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Plant Gene Register

Identification of a *Chlamydomonas reinhardtii* Chloroplast Gene with Significant Homology to Bacterial Genes Involved in Cytochrome *c* Biosynthesis[1]

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As entire chloroplast genomes are sequenced, the structure of chloroplast genes, open reading frames, and spacer regions can be compared. Shimada and Sugiura (1991) identified 11 open reading frames that were conserved in the rice, *Marchantia*, and tobacco chloroplast genomes. These genes have been designated *ycf* genes (Hallick, 1989). There are also 11 additional conserved genes that are thought to encode subunits of a putative chloroplast NADPH dehydrogenase. *Chlamydomonas reinhardtii* offers a suitable experimental system to test the physiological function of these genes because chloroplast genes can be disrupted by insertional mutagenesis. We have located the *ycf5* gene on the *C. reinhardtii* chloroplast genome and report the sequence of this gene and the flanking DNA in this communication (Table I).

C. reinhardtii chloroplast DNA was obtained from strain CC-400, a cell-wall-deficient strain carrying the cw-15 mutation. Chloroplast DNA was obtained by density gradient centrifugation and probed with tobacco DNA probes carrying the *ndhD* gene or the *ycf5* gene. These genes lie adjacent to each other on the tobacco, rice, and *Marchantia* genomes. Although no significant hybridization was observed using the *ndhD*-specific probe, a probe carrying the *ycf5* gene hybridized with both isolated *C. reinhardtii* chloroplast DNA and the cloned *Bam*HI 13 fragment obtained from the *Chlamydomonas* Genetics Center. Sequencing of the BamHI 13 clone indicated that, although the 5′ end of the gene was on the *Bam*HI 13 fragment, part of the gene must be on the adjacent *Bam*HI 17 fragment. The 3′ end of the gene was obtained by polymerase chain amplification of the *Bam*HI 17 fragment using primers from the flanking *Bam*HI 4 and *Bam*HI 13 fragments. The open reading frame that spanned these two BamHI fragments was 1062 bp and would encode a protein of 353 amino acids.

A comparison of the deduced amino acid sequence with other known sequences revealed that the *Chlamydomonas* gene is very homologous to *ycf5* genes found in higher plants. It has 74.5% identity with the *Marchantia ycf5* gene (Ohyama et al., 1986) and 66% identity with the tobacco *ycf5* gene (Shinozaki et al., 1986). In addition, like other *ycf5* genes, it shares a significant homology with some recently discovered bacterial genes (*ccl1* and *helC*) that appear to be involved in Cyt *c* biogenesis (Beckman et al., 1992; Sorokin et al., 1993).

When the *Ccl1* gene in *Rhodobacter capsulatus* is disrupted the cell cannot synthesize any proteins containing a *c*-type Cyt (Biel and Biel, 1990; Beckman et al., 1992), although the cell is still able to synthesize the apoproteins and heme. The defect in cells disrupted at the *Ccl1* locus appears to be in the covalent linkage of the heme to the apoprotein. In *R. capsulatus* the Cyt *c*-containing proteins are periplasmic and the Ccl1 protein is also periplasmic (Beckman et al., 1992). Presently it is thought that proteins cannot cross a

Table I. *Characteristics of the ycf5 gene of C. reinhardtii*

Organism:	*Chlamydomonas reinhardtii.*
Strain:	CC-400 cw-15, mt^+.
Location:	Spanning the *Bam*HI 17 and *Bam*HI 13 fragments of the chloroplast genome. 192 bp are in the *Bam*HI 17 fragment and 870 bp are in the *Bam*HI 13 fragment.
Function:	Unknown in chloroplasts, although *ycf5* genes share significant sequence homology with genes required for Cyt *c* biosynthesis in bacteria.
Techniques of Sequencing:	The *Bam*HI 4 fragment and the *Bam*HI 13 fragment were obtained from the *Chlamydomonas* Genetics Center cloned into pBR313 and pUC19, respectively. The *Bam*HI 17 fragment was obtained by PCR amplification of isolated chloroplast DNA using primers from the *Bam*HI 4 and *Bam*HI 13 fragments. The nucleotide sequence of both strands was determined by the dideoxy chain termination method.
Method of Identification:	By homology of the deduced amino acid sequence with known chloroplast and bacterial genes using the Blitz e-mail server operated by the European Molecular Biology Laboratory.
Features of the DNA Sequence:	The 2249-bp sequence reported here spans the *Bam*HI 17 fragment (731 bp) and 1518 bp of the *Bam*HI 13 fragment from the *Bam*HI 17/*Bam*HI 13 junction to the *Eco*RI site within the *Bam*HI 13 fragment and the beginning of psA-1 exon 1.
Expression Profile:	Probes specific for the *ycf5* gene detected a RNA transcript of approximately 3.3 kb.

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membrane with the heme attached (Thöny-Meyer, 1994). If that is the case, a protein analogous to the *Ccl1* gene product must be present in mitochondria and chloroplasts. Although this function has not been physiologically demonstrated in *C. reinhardtii* or other plants, we have detected a 3.3-kb transcript using a *ycf5*-specific probe, implying that this is a functional chloroplast gene. In the *C. reinhardtii* chloroplast, both Cyt *f* and Cyt *c*-552 (Merchant and Bogorad, 1987) are *c*-type cytochromes and would require the proteins necessary to covalently bind the heme to the apoprotein.

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The GenBank accession number for the sequence reported in this article is U15556.

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